Frequency, diagnosis and management of fungal respiratory infections

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Purpose of review
This review highlights key recent advances in fungal respiratory infections, encompassing developments in epidemiology, diagnostics and management, focussing on *Aspergillus*, *Pneumocystis* and *Cryptococcus* as key pathogens.

Recent findings
Chronic pulmonary aspergillosis complicates existing lung diseases, particularly those associated with cavities or bullae, with a high global disease burden (prevalence estimate >1.1 million following tuberculosis) and significant under diagnosis (using *Aspergillus* IgG antibody). Several new treatment studies have been published (using caspofungin and voriconazole). *Pneumocystis jirovecii* demonstrates airborne transmission between infected and noninfected individuals necessitating isolation, and possibly identifying colonized patients. Early detection of serum cryptococcal antigenaemia in HIV may prevent development of meningitis, reducing morbidity and mortality, and routine testing of serum in community-acquired pneumonia cases in high endemicity areas may be helpful. Respiratory *Aspergillus* antigen and PCR testing is more sensitive than culture or serum testing. A new lateral flow antigen testing device may provide rapid bedside diagnosis of aspergillosis. Azole resistance to *Aspergillus fumigatus* is increasing across Europe.

Summary
The field of fungal respiratory infection continues to evolve and develop, with many recent key advances. Patients, and possibly colonized patients, with *Pneumocystis* require isolation in hospitals and preferably segregation in outpatients. Challenges remain in almost all areas, with further work needed to identify the true burden of *Aspergillus* disease and address the increasing problem of azole resistance.

Keywords
aspergillus, pneumocystis, transplantation

INTRODUCTION

In this short review of the major topic of fungal respiratory infections, we review significant developments over the last 18 months, highlighting important ground-breaking articles.

EPIDEMIOLOGY OF CHRONIC AND ALLERGIC ASPERGILLOSIS

The overall burden of most forms of aspergillosis is not known with any certainty. Estimates have recently been published, not only of alllergic and chronic pulmonary aspergillosis (Fig. 1), but also of some of their underlying diseases, such as asthma and sarcoidosis, in adults.

Chronic pulmonary aspergillosis following tuberculosis

Several efforts have been made to quantify the incidence and prevalence of fungal infections worldwide. Denning *et al.* [1▪,2,3▪] attempted prevalence estimates of chronic pulmonary aspergillosis (CPA), complicating tuberculosis (TB) and sarcoidosis, as well as estimating allergic bronchopulmonary aspergillosis (ABPA), developing a deterministic scenario model, with scooping review methodology for the available literature.

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KEY POINTS

- Epidemiological data surrounding all forms of aspergillosis are expanding and CPA is recognized as having a high global disease burden, complicating existing lung disease, particularly bullous or cavitating, the majority of it being under diagnosed.
- Diagnostic methods are continually evolving with the use of antigen detection and PCR extending to respiratory tract secretions in invasive aspergillosis and the development of near patient testing, the lateral flow device.
- Antifungal agents have not increased in number and increasing azole resistance has been identified. However, new disease targets have been established for existing agents, for example voriconazole and CPA.
- Advances have not been limited to *Aspergillus* with new data regarding transmission of *Pneumocystis* and early detection of cryptococcal infection also being important.

Pulmonary TB continues to be a common problem with 5.8 million notified cases in 2011. With approximately 10% of those surviving pulmonary TB developing CPA, the estimated number of new cases of CPA is 375 000 with a 5-year-period prevalence of 1 170 000 cases. The key assumptions made regarding the frequency of CPA are that 22% of patients are left with a significant cavity after TB, except in Europe in which a figure of 12% has been used (no recent data). In Taiwan and South Africa, computed tomography scanning on completion of therapy revealed about 35% of patients to have a residual cavity. Of these patients, 22% develop CPA, based on UK data from the late 1960s, never evaluated since, and that 2% of those without a cavity develop CPA, never quantified previously. Conversion from annual incidence to 5-year-period prevalence assumed an annual death and surgical resection rate of 15%. Recent data from Korea and Japan have suggested that the first-year mortality after the diagnosis of CPA is much higher (25–30%) [4,5], but the interval between concluding TB treatment and presentation with CPA is uncertain.

Prevalence of sarcoidosis and chronic pulmonary aspergillosis

With respect to sarcoidosis, a similar deterministic scenario model was constructed, with substantially more epidemiological data with some key gaps (such as China, India and Africa) and data conflicts. There is huge variability of pulmonary sarcoidosis prevalence varying from as low as 0.2 per 100 000 in Portugal and Brazil to 64 per 100 000 in Sweden. Overall, 344 000 new cases of sarcoidosis were estimated worldwide annually with a 5-year-period prevalence of 1 238 000 cases. The published series of CPA (mostly based on the radiological finding of an aspergilloma without *Aspergillus* IgG surveillance) indicate an attack rate of 3–12%. Including only those with pulmonary sarcoidosis, a 6% mean CPA rate and a 15% annual mortality rate, there is an estimated burden of 72 000 CPA cases worldwide; 24% and 37% occur in the Americas and Africa, respectively, given the recognized predilection of black people to sarcoidosis [2].

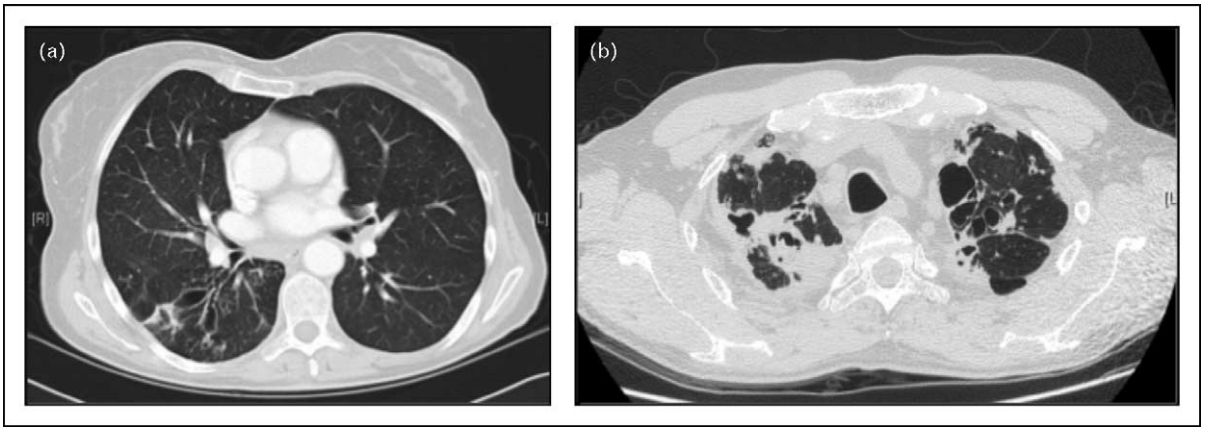

FIGURE 1. Allergic and chronic pulmonary aspergillosis. (a) Right lower lobe bronchiectasis in a woman with allergic bronchopulmonary aspergillosis. There is also some peripheral inflammatory material with a little ground glass opacification, consistent with localized infection. (b) Bilateral chronic pulmonary aspergillosis with cavitation, surrounded by thick infiltrates and on the right some overt pleural thickening posteriorly. At least one bulla in the context of emphysema is seen on the left medially with a small cavity and pericavitary inflammation or fibrosis adjacent. Note no fungal ball or aspergilloma is present, as in ~75% cases of chronic pulmonary aspergillosis.

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Prevalence of asthma and allergic bronchopulmonary aspergillosis in adults

The prevalence of asthma worldwide has been estimated to be 300 million by the 2004 Global Initiative for Asthma (GINA) report, but the basis for these estimates in many locales is far from perfect. Recently, population estimates for asthma have been published from Scotland, 640 000 of 5.1 million [6], and the United States, 24.6 of 305 million in 2009 [7▪▪], allowing reasonable extrapolation of the GINA statistics to produce a new global estimate – 193 million asthmatic (wheezing) adults in 2008. By using primary care records, Anandan [6] also identified that 26–27% of Scottish adults had ever wheezed, 16% of adults had wheezed in the previous 12 months and 13–14% of adults had ever had doctor-diagnosed asthma.

These estimates provide key data from which an estimation of the global burden of ABPA can be made [3▪]. Five time-limited studies of adult asthma referrals to secondary care yielded ABPA rates from 0.7% (Ireland in the late 1980s) to 3.5% (New Zealand in the 1990s), with three other studies (South Africa, Saudi Arabia and China) reporting ABPA rates of 2.3–2.6%. Using a median figure of 2.5%, the global estimate of ABPA is 4 837 000 patients. This could underestimate the true figure because of the short period of referrals compared with a lifetime of disease, or overestimate it because only more symptomatic patients are referred to secondary care. Denning *et al.* [3▪] also attempted to estimate the incidence of CPA complicating ABPA, using highly variable, somewhat selective and subjective retrospective series, calculating a global prevalence of 411 000. Multiple, predominantly community-based, cohort studies are required to validate these modelling estimates.

PNEUMOCYSTIS

Pneumocystis jirovecii is a human-only fungus that has coevolved with us, and recent research has enhanced knowledge of modes of transmission. Outbreaks of *Pneumocystis* pneumonia (PCP) have been noted, especially in renal transplant patients. A link with sudden infant death syndrome (SIDS) has also been made.

Origin of *P. jirovecii* outbreaks and colonization

A recent epidemiological review [8▪] has demonstrated that, although about 20% of adults are colonized, a higher colonization rate is found in children and immunosuppressed adults. About 2 years ago, *P. jirovecii* was demonstrated by quantitative PCR to be present in exhaled air close to patients with PCP, amounts decreasing the further the sampling moved away from patients [9]. At that time, direct transmission, and from whom, had not been formally demonstrated. Using a combination of PCR, molecular typing and transmission mapping, the source of infection of 18 renal transplant patients with PCP over 2 years was traced [10▪▪]. Ten source patients, seven with PCP and three who were simply colonized, were identified. In a separate study, a single molecular type of *P. jirovecii* was found in two outbreaks (Switzerland and Germany), suggesting that some strains may be more transmissible or virulent, or both [11]. The implications are that all hospitalized patients with PCP should be isolated in single rooms and that vulnerable patient groups should be kept away from them. Identifying colonized patients and segregating them from those with known immunosuppression may be beneficial; however, the variability in fungal load over time in those who are colonized is not known. If fungal load is stably high, then segregation, or eradication, is likely to be an effective preventive strategy, and if fungal load varies then it is not. The recent launch of a commercially validated real time PCR test for *P. jirovecii* opens up new diagnostic and management strategies [12▪].

Pneumocystis and sudden infant death syndrome and chronic obstructive pulmonary disease

Pneumocystis has also been linked to SIDS. Postautopsy work from Santiago, Chile, found *P. jiroveii* frequently (82%) in SIDS babies, in whom it was associated with increased expression of MUC5AC and a marked increase in mucus production compared to controls [13]. Increased mucus production likely causes narrowed airways, and potentially airway collapse, leading to death. Similarly, in chronic obstructive pulmonary disease (COPD) *P. jirovecii* colonization is associated with increased expression of matrix metalloproteinase 12 and increased systemic inflammatory response markers [interleukin (IL)-6, IL-8 and tumour necrosis factor-alpha] [8▪]. The role of *P. jirovecii* in COPD is not clear, but *P. jirovecii* is commonly found – 42.1% of explanted lungs in advanced COPD carry the organism [14].

COMMUNITY ACQUIRED PNEUMONIA AND CRYPTOCOCCAL ANTIGENAEMIA

Early detection of cryptococcal antigenaemia (CrAg+) in the absence of meningitis is useful for early diagnosis, targeted treatment and prevention of life-threatening/life-limiting sequelae. Usually, serum antigen testing is only undertaken when meningitis is suspected. Harris *et al.* [15] studied

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the HIV population in rural Thailand, demonstrating that in those patients hospitalized for an acute respiratory illness, 13.1% were CrAg+ on serum sampling, with only TB and rhinovirus being isolated more commonly. Over half the CrAg+ group had no evidence of previous or current cryptococcal infection and 40% of cases may have been cryptococcal pneumonia. Detecting cryptococcal antigenaemia is now much more simple with the introduction of a simple lateral flow antigen test [16▪▪,17▪▪]. Identification of cryptococcal pneumonia, through serum antigenaemia, may provide a valuable early treatment window. A useful case series and review of the characteristics of primary pulmonary cryptococcosis from China has also recently been published [18]. Nodules, some cavitating, some with a halo sign, were common, especially in the lower lobes.

DIAGNOSIS OF ASPERGILLOSIS

Invasive aspergillosis remains difficult to diagnose rapidly enough to save the patient. This reflects its infrequency, initially subtle presentation and multiple patient co-morbidities, along with lack of awareness among multiple medical specialties and poor performance of routine microbiological tests, particularly culture. Significant diagnostic advances have now been made and need to be implemented.

Aspergillus antigen and PCR

Many articles examining the role of *Aspergillus* antigen (galactomannan) and/or *Aspergillus* PCR detection in respiratory fluids have recently been published. Previous work has focused on serum/blood antigen or DNA detection and data generally support their use in the neutropenic haemato-oncology group for diagnosis and/or surveillance, depending on the local incidence of invasive aspergillosis in those not on mould-active prophylaxis, particularly itraconazole and posaconazole. Research efforts have expanded to include respiratory sample testing particularly in immunocompromised nonneutropenic patients, typically those on corticosteroids, critically ill or both. The higher yield of *Aspergillus* antigen and/or PCR from respiratory samples was demonstrated in controlled experiments with infected guinea pigs [19], normal volunteers [20▪], haematology and critical care patients [21], haematology patients [22], solid organ transplants, haematology and other at-risk patients [23], children [24▪], patients critically ill with COPD [25], cystic fibrosis patients [26] and those with allergic and chronic pulmonary aspergillosis [20▪].

Direct comparisons between PCR and *Aspergillus* galactomannan have been done, using either in-house nested PCR or commercial *Aspergillus* assays. Torelli *et al.* [21] demonstrated a superior diagnostic odds ratio (DOR) comparing a commercial PCR assay using bronchoalveolar lavage fluid (BAL) samples to an in-house assay (1120 vs. 350) and aspergillus antigen (DOR 201 at a cut-off of 0.50). A further multicentre prospective trial compared an in-house nested *Aspergillus* PCR with *Aspergillus* antigen in BAL samples from haematological patients. They found good concordance (79%) between positive assays with a combined test sensitivity of 0.55, but a specificity of 1 with a positive predictive value of 100% and an almost infinite DOR [22]. A combined approach to testing appears promising, with positive results for both tests suggesting that pulmonary aspergillosis is very likely with a need for urgent antifungal therapy. A sensitivity of 0.55 is still considerably greater than the published 0.20 observed in BAL cultures in proven invasive aspergillosis. Antifungal therapy with two antifungals reduces the sensitivity of PCR in BAL for the diagnosis of invasive aspergillosis [27], emphasizing the need to acquire the BAL sample rapidly after suspicion of aspergillosis is raised. Separately, D'Haese [23] demonstrated that very high BAL aspergillus antigen values (i.e. >3.0) almost definitively represent infection, whereas a value of less than 0.5 has a 93% sensitivity for ruling it out.

Looking specifically at the comparison of *Aspergillus* PCR with culture yield in sputum from ABPA and CPA, as well as BAL from volunteers and haematological patients, clearly shows the superiority of this technique to culture [20▪]. Low-level PCR positives were found in four out of 11 volunteers undergoing bronchoscopy, confirming that, from a fungal perspective, normal lungs are often not sterile. Additional processing of cystic fibrosis (CF) sputum with sonication increases the culture yield and PCR positivity (signal strength and positive rate) [26]. The value of PCR and *Aspergillus* antigen testing in sputum needs further work, but is promising.

New lateral flow device for *Aspergillus* antigen

A further development is a new, recently developed, point-of-care test for *Aspergillus* antigen by Chris Thornton (Exeter University) [28]. On the basis of lateral flow technology (LFD), and a hybridoma-derived MAb JF5, this test detects an extracellular glycoprotein antigen secreted only during active fungal growth, and takes only 15 min to perform. In interlaboratory studies in infected guinea pigs, the LFD results were usually concordant [29]. In a retrospective study of 39 BAL samples from haematology and solid-organ transplant recipients, sensitivity and specificity for probable invasive

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pulmonary aspergillosis (IPA) were 100 and 81% [positive predictive value (PPV) 71%, negative predictive value (NPV) 100%], respectively, with five patients with possible IPA having positive LFD results [30]. These are promising results that deserve further evaluation.

Invasive aspergillosis in ICU and positive *Aspergillus* cultures

In addition to improved *Aspergillus* diagnostics, work has also focused on developing clinical methodologies for trying to identify patients at risk of invasive disease. An observational international cohort study by Blot *et al.* [31] attempted to validate an established diagnostic algorithm for invasive pulmonary aspergillosis in critically ill patients with positive *Aspergillus* cultures. *Aspergillus* is isolated from 2% of endotracheal aspirates in this population, and distinguishing colonization from invasive aspergillosis is clinically difficult. Current diagnostic criteria [European Organisation for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG)] stratify patients into proven, probable or possible disease according to host, clinical and microbiological features. Within a critical care population, the application of criteria is problematic with probable and possible criteria being validated only in immunocompromised populations. Adapting current criteria, placing less emphasis on host factors and introducing new clinical, radiological and microbiological standards, the algorithm aims to encompass more of the at-risk intensive care population.

When compared with EORTC/MSG criteria, the ICU algorithm performed better [receiver operator curve 76% confidence interval (CI) 67–85% vs. 57% CI 46–68%], and as a means of distinguishing colonization from invasive disease demonstrated a sensitivity of 92% with a specificity of 61% [31]. A low specificity may be acceptable in this context, untreated invasive aspergillosis conferring 100% mortality. The algorithm remains unvalidated for those patients with proven invasive aspergillosis but persistently negative cultures and, although promising, further work is needed before clinical introduction. Cultures are also insensitive, so if patients are thought to be at moderate or high risk, testing endotracheal or BAL samples with *Aspergillus* antigen and/or PCR is a more sensitive means of ruling the diagnosis in or out.

MANAGEMENT

No new antifungal drugs have been licensed, but our understanding of the role of the current drugs has improved slightly for CPA and following lung transplantation. Immune reconstitution syndrome is increasingly recognized, and is difficult to manage.

Treatment of chronic pulmonary aspergillosis

Advances in the management of CPA this year have focused on voriconazole. Voriconazole has now been studied in three prospective trials [31–33], involving 165 patients, to add to older retrospective series. In France, 5 out of 41 patients died during therapy and 7 (17%) were intolerant of voriconazole [33]. The overall response rate at 6 months was 32%, with better responses in subacute invasive (chronic necrotizing) aspergillosis than CPA. In a multicentre study in Japan, 61% responded well with only two patients stopping therapy [34]. A recent study compared caspofungin with micafungin with similar results [35]. These studies extend the options for therapy of CPA beyond itraconazole and micafungin, although no antifungal agent has been demonstrated to have more than a 60% response rate.

Immune reconstitution syndrome during antifungal therapy

Immune reconstitution syndrome (IRS) (formerly IRIS) as a differential diagnosis for failure of treatment in invasive fungal infection was explored in an illustrated case review by Perfect [36]. Being described as restoration of host immunity with an associated aggressive proinflammatory response following a period of immune suppression, IRS has been described in multiple patient populations including opportunistic mycoses and is probably underdiagnosed in this patient group. Predominantly a T-lymphocyte-driven process, its development is thought to be driven by a combination of interacting host, pathogen and antifungal factors. All classes of antifungal therapy have been linked to immunomodulation, and certain fungi, including *Cryptococcus neoformans*, are thought to modulate T-cell response. The impact of host genetics and underlying immune competence may also play a role.

Diagnosing IRS in invasive fungal infection, however, remains difficult. Establishment of the absence of intrinsic or acquired antifungal resistance, therapeutic drug levels and the possibility of mixed infection should all be considered prior to diagnosis. Criteria have been established, encompassing new radiological changes, worsening symptoms despite appropriate antifungal therapy and the presence of persistently negative cultures or stable/reduced levels of fungal markers to aid diagnosis. As yet, no defined biomarker is available that will diagnose IRS or distinguish it from recurrent infection. Treatment is not always necessary, but corticosteroids and nonsteroidal drugs are used. Identifying

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and treating IRS in the context of invasive fungal infection is important to prevent continued deterioration, exposure to increasingly toxic antifungal regimens and development of refractory disease. Ultimately, recognition of IRS in invasive fungal infection will improve long-term outcomes for patients.

Antifungal prophylaxis in lung transplantation

Prevention of fungal infection, particularly invasive aspergillosis, after lung transplantation is difficult and no consensus guidelines exist. Long-term triazole prophylaxis is often used within this patient group, but evidence is lacking. Koo *et al.* [37] identified bilateral lung transplantation and positive perioperative fungal cultures as significant risk factors for postoperative invasive fungal disease. A tailored regimen of inhaled amphotericin B along with additional micafungin for double-lung recipients and 3–6 months of oral azole tailored to specific fungal isolates for those with positive perioperative fungal cultures was instituted in response. Promisingly, a sharp decrease in invasive candidiasis and invasive aspergillosis was observed at 1 year, and only 19% of patients received systemic antifungal therapy. One-year rates of invasive aspergillosis were comparable to or lower than those reported by other centres using a standard triazole regimen. This regimen may have the potential to successfully reduce the incidence of invasive fungal disease, while sparing the patient significant triazole associated side-effects and associated healthcare costs.

AZOLE RESISTANCE

Despite the above advances in management strategies across the disease spectrum, azole resistance remains a problem. First described in the late 1980s from Californian isolates, itraconazole resistance in *Aspergillus fumigatus* has exponentially increased, primarily in northern Europe [38▪]. In the Netherlands, the prevalence of azole resistance varies from 0.8 to 9.4% between centres [39▪▪], and in Denmark, 4.5% of *A. fumigatus* isolates obtained from 133 patients with CF were triazole resistant, most harbouring the TR_{34}/L98H mutation [40]. Similarly, 4.6–8% of French patients with CF demonstrated resistant isolates [41,42]. Resistance is also reported from France, Spain, Belgium, Germany, Japan, China and India. Itraconazole resistance is not confined to *Aspergillus fumigatus*, also being documented in a few *A. niger* and *A. terreus* isolates. Although regular susceptibility testing of all clinical strains should be undertaken, only a few laboratories do so, and consequently resistance rates are probably underreported. The first reports of direct azole resistance detection via direct molecular methods, without culture, have now appeared [20▪,43▪], which presages more rapid answers for clinicians although the number of resistance mechanisms now described is large.

CONCLUSION

We have summarized estimates of the burden of disease of sarcoidosis, asthma, ABPA and CPA, the transmissibility of *Pneumocystis* and its association with chronic respiratory disease and inflammation in COPD, improved diagnostics for aspergillosis with recognition of the poor performance of culture against galactomannan antigen detection and PCR, and better treatment for CPA, IRS and azole resistance. The field continues to evolve with significant progress being made in several areas.

Acknowledgements

None.

Conflicts of interest

G.E.H. has no conflicts of interest. D.W.D. holds founder shares in F2G Ltd, a University of Manchester spin-out company, and has current grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, the European Union and AstraZeneca. He acts as an advisor/consultant to F2G and Myconostica (now part of Lab21 Group) and T2Biosystems as well as other companies over the last 5 years, including Pfizer, Schering Plough (now Merck), Nektar, Astellas and Gilead. He has been paid for talks on behalf of Merck, Astellas, GSK, Novartis, Merck, Dainippon and Pfizer.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ▪ of special interest
- ▪▪ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

1. Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary ▪ aspergillosis as a sequel to tuberculosis. Bull WHO 2011; 89:864–872.

The first attempt to estimate the burden of this CPA worldwide, with a surprisingly large number of people affected (nearly 1.2 million). As rates of TB fall, CPA could also slowly fall, although risk remains for years.

2. Denning DW, Pleuvry A, Cole DC. Global burden of chronic pulmonary aspergillosis complicating sarcoidosis. Eur Respir J 2012. [Epub ahead of print]
3. Denning DW, Pleuvry A, Cole DC. Global burden of allergic bronchopulmon- ▪ ary aspergillosis in adults with asthma and its complication chronic pulmonary aspergillosis. Med Mycol 2012. [Epub ahead of print]

The first attempt to estimate ABPA (and CPA complicating it), as well as a revised global burden of asthma. Total ABPA burden is estimate as 4.8 million people, with probably marked regional variations, probably lower than this in the United States, and higher in India, for example.

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4. Nam HS, Jeon K, Um SW, *et al.* Clinical characteristics and treatment outcomes of chronic necrotizing pulmonary aspergillosis: a review of 43 cases. Int J Infect Dis 2010; 14:e479–e482.
5. Ohba H, Miwa S, Shirai M, *et al.* Clinical characteristics and prognosis of chronic pulmonary aspergillosis. Respir Med 2012; 106:724–729.
6. Anandan C, Gupta R, Simpson CR, *et al.* Epidemiology and disease burden from allergic disease in Scotland: analyses of national databases. J R Soc Med 2009; 102:431–442.
7. ■■ Akinbami LJ, Moorman JE, Liu X. Asthma prevalence, healthcare use, and mortality: United States, 2005–2009. Natl Health Stat Report 2011; 12:1–14.

Important data on the burden of asthma in the United States today. In 2009, asthma prevalence was 8.2% of the US population (24.6 million people). In 2008, persons with asthma missed 10.5 million school days and 14.2 million work days due to their asthma. In 2007, there were 1.75 million asthma-related emergency department visits and 456 000 asthma hospitalizations.

8. ■ Morris A, Norris KA. Colonization by *Pneumocystis jirovecii* and its role in disease. Clin Microbiol Rev 2012; 25:297–317.

Really useful review summarizing many aspects of the biology and impact of *Pneumocystis*. Particular focus on *Pneumocystis* and COPD exacerbations.

9. Choukri F, Menotti J, Sarfati C, *et al.* Quantification and spread of *Pneumocystis jirovecii* in the surrounding air of patients with *Pneumocystis* pneumonia. Clin Infect Dis 2010; 51:259–265.
10. ■■ Le Gal S, Damiani C, Rouillé A, *et al.* A cluster of *Pneumocystis* infections among renal transplant recipients: molecular evidence of colonized patients as potential infectious sources of *Pneumocystis jirovecii*. Clin Infect Dis 2012; 54:e62–e71.

Direct evidence of transmission from colonized patients to cases, implicating not only those with PCP, but those who excrete the organism, potentially of profound importance in populations not on prophylaxis.

11. Sassi M, Ripamonti C, Mueller NJ, *et al.* Outbreaks of Pneumocystis pneumonia in two renal transplant centers linked to a single strain of Pneumocystis: implications for transmission and virulence. Clin Infect Dis 2012; 54:1437–1444.
12. ■ Hauser PM, Bille J, Lass-Flörl C, *et al.* Multicenter, prospective clinical evaluation of respiratory samples from subjects at risk for *Pneumocystis jirovecii* infection by use of a commercial real-time PCR assay. J Clin Microbiol 2011; 49:1872–1878.

First commercial evaluation of a commercial *Pneumocystis* real-time PCR assay. In 110 samples, PCR sensitivity, specificity, PPV and NPV were 93, 90, 65 and 98%. Of the nine PCR-positive subjects without PCP, one later developed PCP.

13. Vargas SL, Ponce CA, Gallo M, *et al.* Near-universal prevalence of Pneumocystis and associated increase in mucus in the lungs of infants with sudden unexpected death. Clin Infect Dis 2013; 56:171–179.
14. Sivam S, Sciurba FC, Lucht LA, *et al.* Distribution of *Pneumocystis jirovecii* in lungs from colonized COPD patients. Diagn Microbiol Infect Dis 2011; 71:24–28.
15. Harris JR, Lindsley MD, Henchaichon S, *et al.* High prevalence of cryptococcal infection among HIV-infected patients hospitalized with pneumonia in Thailand. Clin Infect Dis 2012; 54:e43–e50.
16. ■■ Jarvis JN, Percival A, Bauman S, *et al.* Evaluation of a novel point-of-care cryptococcal antigen test on serum, plasma, and urine from patients with HIV-associated cryptococcal meningitis. Clin Infect Dis 2011; 53:1019–1023.

Of critical importance to reducing the high mortality from cryptococcal meningitis throughout Africa, this is a highly sensitive 10-min, simple, inexpensive bedside assay. It can be used on urine as well, to screen, although blood confirmation is also required.

17. ■■ Lindsley MD, Mekha N, Baggett HC, *et al.* Evaluation of a newly developed lateral flow immunoassay for the diagnosis of cryptococcosis. Clin Infect Dis 2011; 53:321–325.

See [16■■]

18. Qu Y, Liu G, Ghimire P, *et al.* Primary pulmonary cryptococcosis: evaluation of CT characteristics in 26 immunocompetent Chinese patients. Acta Radiol 2012; 53:668–674.
19. Lengerova M, Kocmanova I, Racil Z, *et al.* Detection and measurement of fungal burden in a guinea pig model of invasive pulmonary aspergillosis by novel quantitative nested real-time PCR compared with galactomannan and (1,3)-β-D-glucan detection. J Clin Microbiol 2012; 50:602–608.
20. ■ Denning DW, Park S, Lass-Florl C, *et al.* High-frequency triazole resistance found in nonculturable *Aspergillus fumigatus* from lungs of patients with chronic fungal disease. Clin Infect Dis 2011; 52:1123–1129.

First demonstration of direct detection of azole resistance in a clinical specimen, without a positive culture, for any fungal infection. It is challenging technology, partly because of multiple mechanisms of resistance.

21. Torelli R, Sanguinetti M, Moody A, *et al.* Diagnosis of invasive aspergillosis by a commercial real-time PCR assay for *Aspergillus* DNA in bronchoalveolar lavage fluid samples from high-risk patients compared to a galactomannan enzyme immunoassay. J Clin Microbiol 2011; 49:4273–4278.
22. Reinwald M, Spiess B, Heinz WJ, *et al.* Diagnosing pulmonary aspergillosis in patients with hematological malignancies: a multicentre prospective evaluation of an Aspergillus PCR assay and a galactomannan ELISA in bronchoalveolar lavage samples. Eur J Haematol 2012; 89:120–127.
23. D'Haese J, Theunissen K, Vermeulen E, *et al.* Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. J Clin Microbiol 2012; 50:1258–1263.
24. ■ de Mol M, de Jongste JC, van Westreenen M, *et al.* Diagnosis of invasive pulmonary aspergillosis in children with bronchoalveolar lavage galactomannan. Pediatr Pulmonol 2012. [Epub ahead of print]

Important new data on respiratory sample diagnosis in children. Sensitivity, specificity, PPV and NPV of BAL galactomannan for a diagnosis of proven and probable IPA ($n = 41$) were 82.4, 87.5, 82.4 and 87.5%, respectively.

25. He H, Ding L, Sun B, *et al.* Role of galactomannan determinations in bronchoalveolar lavage fluid samples from critically ill patients with chronic obstructive pulmonary disease for the diagnosis of invasive pulmonary aspergillosis: a prospective study. Crit Care 2012; 16:R138.
26. Baxter CG, Jones AM, Webb K, Denning DW. Homogenisation of cystic fibrosis sputum by sonication: an essential step for *Aspergillus* PCR. J Microbiol Methods 2011; 85:75–81.
27. Reinwald M, Hummel M, Kovalevskaya E, *et al.* Therapy with antifungals decreases the diagnostic performance of PCR for diagnosing invasive aspergillosis in bronchoalveolar lavage samples of patients with haematological malignancies. J Antimicrob Chemother 2012; 67:2260–2267.
28. Thornton C, Johnson G, Agrawal S. Detection of invasive pulmonary aspergillosis in haematological malignancy patients by using lateral-flow technology. J Vis Exp 2012; 22:pii:3721. doi:10.3791/3721.
29. Wiederhold NP, Najvar LK, Bocanegra R, *et al.* Interlaboratory and interstudy reproducibility of a novel lateral-flow device and influence of antifungal therapy on the detection of invasive pulmonary aspergillosis. J Clin Microbiol 2013; 51:459–465.
30. Hoenigl M, Koidl C, Duettman W, *et al.* Bronchoalveolar lavage lateral-flow device test for invasive pulmonary aspergillosis diagnosis in haematological malignancy and solid organ transplant patients. J Infect 2012; 65:588–591.
31. Blot SI, Taccone FS, Van den Abeele AM, *et al.* A clinical algorithm to diagnose invasive pulmonary aspergillosis in critically ill patients. Am J Respir Crit Care Med 2012; 186:56–64.
32. Kohno S, Izumikawa K, Ogawa K, *et al.* Intravenous micafungin versus voriconazole for chronic pulmonary aspergillosis: a multicenter trial in Japan. J Infect 2010; 61:410–418.
33. Cadranel J, Philippe B, Hennequin C, *et al.* Voriconazole for chronic pulmonary aspergillosis: a prospective multicenter trial. Eur J Clin Microbiol Infect Dis 2012; 31:3231–3239.
34. Saito T, Fujiuchi S, Tao Y, *et al.* Efficacy and safety of voriconazole in the treatment of chronic pulmonary aspergillosis: experience in Japan. Infection 2012; 40:661–667.
35. Kohno S, Izumikawa K, Yoshida M, *et al.* A double-blind comparative study of the safety and efficacy of caspofungin versus micafungin in the treatment of candidiasis and aspergillosis. Eur J Clin Microbiol Infect Dis 2012. [Epub ahead of print]
36. Perfect JR. The impact of the host on fungal infections. Am J Med 2012; 125:S39–S51.
37. Koo S, Kubiak DW, Issa NC, *et al.* A targeted peritransplant antifungal strategy for the prevention of invasive fungal disease after lung transplantation: a sequential cohort analysis. Transplantation 2012; 94:281–286.
38. ■ Howard SJ, Arendrup MC. Acquired antifungal drug resistance in *Aspergillus fumigatus*: epidemiology and detection. Med Mycol 2011; 49 (Suppl 1): S90–S95.

Useful review of this rapidly expanding topic. Different mechanisms of resistance are reviewed, as well as geography, and a clear distinction is shown between resistance acquired during therapy and primary resistance from the environment.

39. ■■ Van der Linden JW, Snelders E, *et al.* Clinical implications of azole resistance in *Aspergillus fumigatus*, The Netherlands 2007–2009. Emerg Infect Dis 2011; 17:1846–1854.

Discusses the clinical relevance of azole resistance in patients with invasive disease – 88% mortality. Most cases carried the TR/L98H resistance mutation, which confers itraconazole and voriconazole resistance and reduces susceptibility to posaconazole.

40. Mortensen KL, Jensen RH, Johansen HK, *et al. Aspergillus* species and other moulds in respiratory samples from patients with cystic fibrosis: a laboratory-based study with focus on *Aspergillus fumigatus* azole resistance. J Clin Microbiol 2011; 49:2243–2251.
41. Morio F, Aubin GG, Danner-Boucher I, *et al.* High prevalence of triazole resistance in *Aspergillus fumigatus*, especially mediated by TR/L98H, in a French cohort of patients with cystic fibrosis. J Antimicrob Chemother 2012; 67:1870–1873.
42. Burgel PR, Baixench MT, Amsellem M, *et al.* High prevalence of azole-resistant *Aspergillus fumigatus* in adults with cystic fibrosis exposed to itraconazole. Antimicrob Agents Chemother 2012; 56:869–874.
43. ■ Spiess B, Seifarth W, Merker N, *et al.* Development of novel PCR assays to detect azole resistance-mediating mutations of the *Aspergillus fumigatus* cyp51A gene in primary clinical samples from neutropenic patients. Antimicrob Agents Chemother 2012; 56:3905–3910.

Describes direct detection of resistance from positive PCR samples obtained in serum in haematology patients – a step toward direct detection of resistance.

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